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Phosphorylation of the regulatory β-subunit of protein kinase CK2 by checkpoint kinase Chk1: identification of the in vitro CK2β phosphorylation site

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Abstract The regulatory β-subunit of protein kinase CK2 mediates the formation of the CK2 tetrameric form and it has functions independent of CK2 catalytic subunit through interaction with several intracellular proteins. Recently, we have shown that CK2β associates with the human checkpoint kinase Chk1. In this study, we show that Chk1 specifically phosphorylates in vitro the regulatory β-subunit of CK2. Chymotryptic peptides and mutational analyses have revealed that CK2β is phosphorylated at Thr213. Formation of a stable complex between CK2β and Chk1 is not affected by the modification of Thr213 but it does require the presence of an active Chk1 kinase.

Keywords: Protein kinase CK2; Chk1 kinase; Phosphorylation; Mass spectrometry

1. Introduction

Protein kinase CK2 is a constitutively active enzyme, highly conserved in all eukaryotic species so far investigated and essential for viability.

The mammalian heterotetrameric form of CK2 consists of two catalytic subunits (α and/or α′) and two regulatory β-subunits. A significant body of evidences indicates that CK2 regulates, through protein interaction and/or phosphorylation, a wide variety of proteins involved in the control of various aspects of cellular function, i.e., nucleic acid and protein synthesis, cell cycle control, cell proliferation and tumorigenesis [1–3]. Biosynthetic labeling studies have shown that CK2β is synthesized in excess of CK2α and that the formation of CK2β dimers precedes the interaction with the catalytic subunits for the formation of the CK2 tetrameric form [4]. Moreover, mounting evidences have suggested that CK2β exists in cells in the absence of CK2 catalytic subunits indicating that this protein has independent functions exerted through complex formation with nuclear and cytoplasmic proteins [2,5,6]. Recently, we have identified a new CK2β interacting protein: the checkpoint kinase Chk1 [7]. Chk1 is a serine/threonine protein kinase first identified in *S. pombe* as an essential protein kinase for G2 cell cycle arrest induced by DNA damage [8]. Chk1, structurally unrelated but functionally acting in concert with Chk2, is a key enzyme which coordinates cell cycle progression and preserves genome integrity (for review see [9]). Targeted disruption of mammalian Chk1 in mice shows that embryos depleted of Chk1 exhibit morphological abnormalities in nuclei at the blastocyst stage and indicate that Chk1 is indispensable for cell proliferation and survival through the preservation of the G2 checkpoint [10,11]. Chk1 appears to be an active enzyme even in the absence of DNA damage [12,13]. Cell cycle perturbation induced by DNA damage or stalled replication leads to further activation of Chk1 kinase, which in turn targets downstream proteins involved in the response to genotoxic stress. To better elucidate the precise mechanisms involved in Chk1 kinase regulation, we demonstrated that the functional interaction between Chk1 and CK2β leads to enhanced Chk1 kinase activity with respect to Cdc25C phosphorylation [7].

In this report, we show evidence that the native and recombinant human Chk1 kinase phosphorylates the regulatory β-subunit of CK2. Moreover, Chk1 does not target the catalytic subunit of CK2 nor the CK2 holoenzyme indicating the specificity of Chk1-catalyzed CK2β phosphorylation. In the attempt to map CK2β phosphorylation site we have identified, as a recognition residue, Thr213 which maps to the C-terminal domain of CK2β protein.

2. Materials and methods

2.1. Plasmid constructs

Human HA-tagged Chk1 plasmid was a gift from Clare Mc Gowan (San Diego, CA, USA). CK2β-MycHis plasmid was cloned into pcDNA3.1 vector (Invitrogen) as described previously [7]. CK2β T213A-MycHis mutant was generated by PCR mutagenesis from wild-type pcDNA3.1 CK2β-MycHis using the QuickChange site-directed mutagenesis kit (Stratagene). The primers were the following: forward primer 5′-GAGCCCAGTCAAGGCGATTCGCTGA-3′, reverse primer 5′-TCAGCGAATCGCCTTGACTGGGCTC-3′. The kinase-inactive mutant of Chk1 (HA-Chk1KD) was generated substituting Asp130 with Ala using as a template wild-type HA-Chk1-expressing plasmid and the following primers: forward primer 5′-ATTGGAATAACTCACAGGGCTATTAAACCAGAAAATCTT-3′, reverse primer 5′-AAGATTTTCTGGTTTAATAGCCCTGTGAGTTATTCCAAT-3′. In all the constructs, the correct sequence and orientation were verified by DNA sequencing.

2.2. Cell culture and transfection procedure

Cos-1 cell line was grown in Dulbecco's MEM (Gibco) supplemented with 10% (v/v) fetal bovine serum at 37 °C with 5% CO_2. Cells

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were transfected using FuGene transfection reagent (Roche) according to the manufacturer's instructions. Cells were harvested after 48 h from the beginning of transfection.

2.3. Preparation of cell extracts

Protein extracts from cells transiently overexpressing Chk1 and CK2β were prepared as previously described [6] and subsequently utilized for various experiments as indicated in Section 3.

2.4. Antibodies, immunoprecipitation and immunoblotting

The anti-Chk1 monoclonal antibody (G-4) was purchased from Santa Cruz, the anti-HA monoclonal antibody was purchased from Babco. Protein kinase CK2 α-subunit was detected with a mouse monoclonal antibody (1AD9), CK2 β-subunit was detected with a mouse monoclonal antibody (6D5) both purchased from Calbiochem. Rabbit polyclonal anti-HA antibody (Y-11) and rabbit polyclonal anti-Myc antibody (A-14) were purchased from Santa Cruz. Rabbit polyclonal anti-CK2β antibody was obtained by immunizing rabbits against the native protein.

Immunoprecipitation experiments and protein visualization were performed essentially as described previously [6,7].

2.5. Purification of recombinantly expressed proteins

The wild-type form of CK2β, the deletion mutant CK2β (1–155) and the mutant CK2β T213A were cloned into pT7-7 vector and expressed into the BL21(DE3) bacterial strain. The expression and purification of these proteins were performed as described previously [7]. CK2α and CK2 holoenzyme were expressed and purified essentially as described in [14]. Recombinant human GST-hChk1 was purchased from Upstate Biotechnology, while GST-Cdc25C$_{200–256}$ was expressed and purified according to [7].

2.6. Protein kinase assays

Recombinant CK2α, CK2β, and CK2β mutants, CK2 holoenzyme or GST-Cdc25C$_{200–256}$ were incubated with recombinant GST-hChk1 in kinase buffer containing 50 mM Tris–HCl, pH 7.4, 1 mM DTT, 10 mM $MgCl_2$, 50 μM ATP, 10 μCi [γ-^{32}P]ATP and 100 μM sodium orthovanadate. Reaction mixtures (30 μl) were incubated 30 min at 30 °C and analyzed by SDS–PAGE after adding SDS sample buffer. The amounts of enzymes and proteins used in the assays are indicated in the figure legends. In some experiments heparin from bovine lung (Sigma) was added to a final concentration of 0.05 μg/μl. Protein extracts from cells co-transfected with HA-Chk1 and CK2β-MycHis or CK2β T213A-MycHis were utilized for co-immunoprecipitation experiments using monoclonal anti-Chk1 antibody. Chk1 kinase assay was performed in kinase buffer (final volume 60 μl) for 30 min at 30 °C. Reactions were stopped by addition of SDS sample buffer. Phosphorylated proteins were separated by SDS–PAGE, transferred to a PVDF membrane and visualized by autoradiography. The quantification of the phosphate incorporated into the substrate targets was done by scintillation counting (Hewlett-Packard) of the excised radioactive bands. The kinase-inactive mutant of Chk1 (HA-Chk1KD) was tested in an in vitro kinase assay (using GST-Cdc25C$_{200–256}$ as a substrate) after immunoprecipitation of Chk1 with polyclonal anti-HA antibody from cells transfected with HA-Chk1KD plasmid.

2.7. Sample preparation for mass spectrometric analysis

5 μg CK2β was in vitro phosphorylated by Chk1 in a non-radioactive kinase assay. Two gel bands, each representing 2.5 μg of CK2β, were excised and the proteins were reduced in the gel using 10 mM DTT in 0.1 M NH_4HCO_3 for 45 min at 56 °C. The proteins were then alkylated using 55 mM iodoacetamide in 0.1 M NH_4HCO_3 for 30 min. Subsequently, in gel digestion was performed at 37 °C overnight using 0.375 μg chymotrypsin (Worthington) in 50 mM NH_4HCO_3 as described previously [15].

2.8. Liquid chromatography electrospray ionization tandem mass spectrometry

In order to identify the CK2β residue(s) phosphorylated by Chk1, online liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) analysis was performed. The analysis and database searching was performed as described in [16] with the following modification: peptides were eluted using a linear gradient of 0–50% of buffer B (90% (v/v) acetonitrile, 1% (v/v) formic acid, 0.6% (v/v) acetic acid and 0.005% (v/v) heptafluorobutyric acid) in 40 min.

3. Results

3.1. Checkpoint kinase Chk1 specifically phosphorylates the regulatory β-subunit of protein kinase CK2

With an in vitro kinase assay using bacterially expressed CK2β we could confirm, as previously observed [7], that CK2β is phosphorylated by recombinant Chk1 (Fig. 1A, lane 2) with a stoichiometry of phosphate incorporation of 1.1 pmol/pmol of CK2β substrate. A kinase assay performed in the presence of equimolar amounts of CK2β and GST-Cdc25C$_{200–256}$ showed that despite the fact that Cdc25C is the favorite substrate for Chk1 kinase, CK2β is a competitive, albeit weak, substrate for Cdc25C: the phosphate incorporation in Cdc25C substrate is reduced by 27% as compared to when CK2β is absent (Fig. 1A, lanes 3 and 4). A phosphorylation assay with Chk1 performed in the presence of constant amounts of CK2β (i.e., 100 pmol) confirmed the previous observation inasmuch that the phosphorylation of Cdc25C increases in relation to increasing amounts of Cdc25C (i.e., from 50 pmol up to 300 pmol), while the phosphorylation of CK2β decreases concomitantly (Fig. 1B, lanes 1–4). Similarly to the experiment shown in Fig. 1A, when increasing amounts of CK2β (i.e., from 50 pmol up to 200 pmol) were added to a fixed amount of Cdc25C (100 pmol), we did not observe a significant decrease of Cdc25C phosphorylation (results not shown). Next, we wanted to verify whether Chk1 targets specifically and exclusively the regulatory subunit of protein kinase CK2. We performed an in vitro kinase assay using recombinant CK2α and CK2 holoenzyme as additional Chk1 potential substrate targets. A specific inhibitor of CK2, heparin, was used in some phosphorylation reactions in order to effectively block CK2 autophosphorylation activity. Heparin is a highly specific polyanionic inhibitor of CK2 but not of Chk1 kinase (Fig. 1C, lanes 1 and 2, [7]). As shown in Fig. 1D, Chk1 kinase specifically phosphorylates CK2β-subunit (Fig. 1D lane 8) but does not phosphorylate the catalytic α-subunit of CK2 (Fig. 1D, lanes 3, 9 and 10). Interestingly, the quantification of incorporated radioactive phosphate revealed that CK2β within the holoenzyme no longer represents a Chk1 substrate target (Fig. 1D, lanes 4, 5 and 11). In order to verify the ability of the native form of Chk1 kinase to phosphorylate CK2β, ectopically expressed HA-tagged Chk1 was co-immunoprecipitated with ectopically expressed CK2β-MycHis using a monoclonal anti-Chk1 antibody (Fig. 2). The incubation of the immune complex with a radioactive mix in an in vitro kinase assay led to the phosphorylation of a protein that appeared to be the exogenously expressed CK2β-subunit as revealed by probing the Western blot membrane with a polyclonal anti-CK2β antibody.

3.2. Chk1 kinase phosphorylates the regulatory β-subunit of CK2 at its C-terminal domain

The determination of substrate specificity for human Chk1 by the oriented peptide library approach [17] has revealed that Chk1 kinase has a strong preference for basic residues at the −3 position and hydrophobic residues at the +1 position relative to the phosphorylated residue. Interestingly, a closer look at the consensus sequence identified in several Chk1 substrate targets has revealed that for some proteins, i.e., p53 and Brca-1, there is a lack of a common motif. Taking that into consideration, we attempted to identify all possible Chk1-catalyzed CK2β phosphorylation sites (Fig. 3A). As shown in

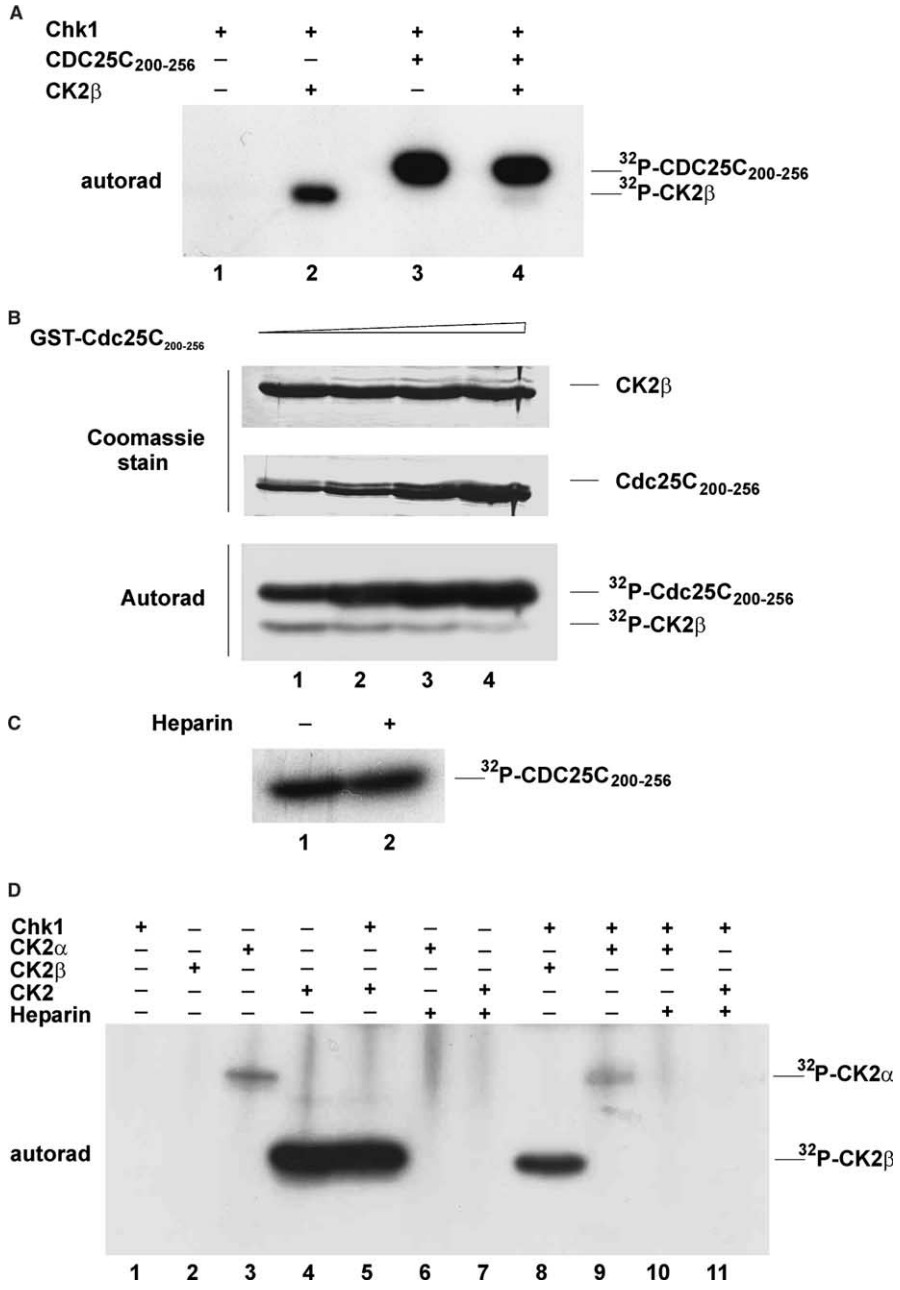

Fig. 1. Chk1 kinase phosphorylates specifically CK2β in vitro. (A) In vitro kinase assay of GST-tagged $Cdc25C_{200–256}$ (lanes 3 and 4) and recombinant CK2β (lanes 2 and 4) by recombinant GST-Chk1 kinase. The kinase assay was performed in the presence of equimolar amounts (96 pmol) of GST-$Cdc25C_{200–256}$ and CK2β and 2 pmol recombinant GST-Chk1 as a kinase source. (B) Chk1 kinase assay performed in the presence of increasing amounts of GST-$Cdc25C_{200–256}$ (lane 1, pmol; lane 2, pmol; lane 3, pmol; and lane 4, 300 pmol) and equal amounts of CK2β (lanes 1–4, 100 pmol). (C) Chk1 kinase assay performed in the absence or in the presence (lanes 1 and 2, respectively) of 50 ng/μl heparin using as substrate target GST-$Cdc25C_{200–256}$. (D) Autoradiograph showing the specific phosphorylation of CK2β by Chk1 kinase. Lanes 1, 2, 3 and 4 represent control experiments where proteins were incubated in the presence of a radioactive mix. Lanes 6 and 7 represent also control experiments where CK2α and CK2 holoenzymes were incubated in the presence of a radioactive mix containing heparin. Lanes 5, 8 and 9 show phosphorylation experiments where CK2 holoenzyme, CK2β and CK2α, respectively, were incubated in the presence of Chk1 kinase. Lanes 10 and 11 are like lanes 9 and 5, respectively, where samples were incubated in the presence of heparin. The data are representatives of three independent experiments.

Fig. 1D, Chk1 kinase phosphorylates the regulatory β-subunit of CK2 as an isolated subunit but not when CK2β is in complex with CK2α. Looking at the crystal structure of the human CK2 holoenzyme [5], we speculated that CK2β phosphorylation site could reside in the C-terminal domain of CK2β which, within the CK2 holoenzyme, makes contacts with the distal β-subunit: a stretch unaccessible for Chk1-catalyzed CK2β phosphorylation. The possibility was tested by an in vitro kinase assay using recombinant CK2β wild-type or truncated CK2β (1–155) forms (Fig. 3B) as substrates and recombinant Chk1 as a kinase source. As predicted, the C-terminal truncated CK2β (1–155) did not show a phosphorylation comparable to the one observed with CK2β wild-type (Fig. 3C, lanes 2 and 3).

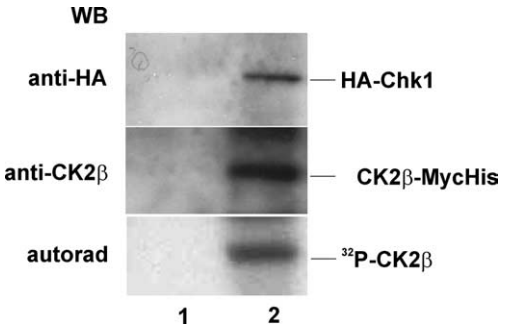

Fig. 2. Specific phosphorylation of CK2β by Chk1 kinase in cells transiently overexpressing both proteins. Cos-1 cells were transfected with HA-Chk1 and CK2β-MycHis expressing plasmids. Total protein lysate was subjected to immunoprecipitation with monoclonal anti-Chk1 antibody. The precipitates were assayed for Chk1 kinase activity using the co-precipitating CK2β as substrate target (lane 2). Lane 1 represents a control experiment performed in the presence of control IgG antibodies. Samples were resolved by SDS–PAGE, analyzed by Western blot and by autoradiography. Chk1 was detected with a polyclonal anti-HA antibody and CK2β with an affinity-purified polyclonal antibody.

3.3. The regulatory β-subunit of CK2 is phosphorylated by Chk1 at Thr213

Based on the previous results, the exact CK2β phosphorylation site was identified by mass spectrometry. Theoretical enzymatic cleavages of CK2β using either of the serine proteases, trypsin or chymotrypsin, were performed. The resulting candidate peptides between residues 181 and 215 are shown in Table 1. Initial analysis of tryptic or chymotryptic in-gel digested peptides were performed using matrix assisted laser desorption/ionization (MALDI) mass spectrometry. A peptide with the m/z value of 1007.6 Da was observed in the chymotryptic peptide mass map which could correspond to the 208–215 peptide carrying one phosphorylation (data not shown). Further investigations were performed using LC-ESI-MS/MS in order to determine the exact phosphorylation site in the 1007.6 Da peptide. The search for phosphopeptides led to the identification of the 208-K**S**PVK**T**IR-215 peptide with a single phosphorylation based on the observed mass (data not shown). The MS/MS spectrum showing the fragmentation of the doubly charged precursor ion (m/z 504.77) corresponding to the phosphorylated peptide of interest was manually inter-

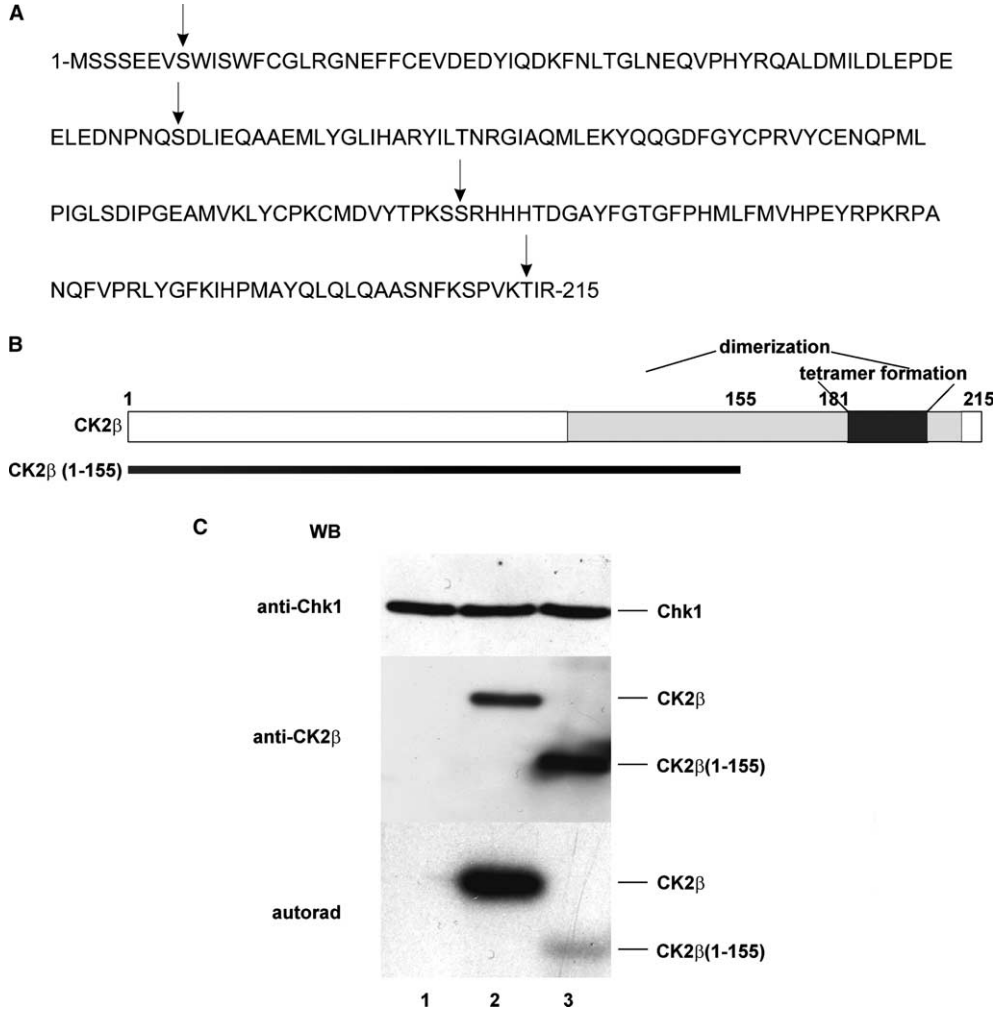

Fig. 3. Chk1 phosphorylates CK2β in the C-terminal domain. (A) Primary sequence of human CK2β. Arrows indicate the hypothetical phosphorylation sites targeted by Chk1 according to Chk1 consensus sequence. (B) Schematic representation of the constructs encoding CK2β wild-type (upper) or a deletion mutant (lower). (C) In vitro kinase assay of bacterially expressed CK2β wild-type (lane 2) and CK2β (1–155) deletion mutant (lane 3) using recombinant active GST-Chk1. Proteins were separated by SDS–PAGE, transferred to PVDF membrane and exposed to film for visualizing phosphorylated CK2β. Proteins were revealed by probing the blots with anti-CK2β and anti-Chk1 antibodies.

Table 1
Candidate peptides containing potential target serine or threonine residues based on theoretical enzymatic cleavages using trypsin (upper) and chymotrypsin (lower), respectively

MH^+	Residues	Sequence
Trypsin		
389.25	213–215	**T**IR
430.27	209–212	**S**PVK
1960.01	192–208	IHPMAYQLQLQAA**S**NFK
Chymotrypsin		
928.59	208–215	K**S**PVK**T**IR
1119.58	198–207	QLQLQAA**S**NF
1960.01	191–207	KIHPMAYQLQLQAA**S**NF
2029.16	198–215	QLQLQAA**S**NFK**S**PVK**T**IR

MH^+ is the monoisotopic mass over charge (m/z) value. Serines (**S**) and threonines (**T**) are in bold.

preted (Fig. 4). Threonine 213 appeared to be the phosphorylated residue, since the mass difference of 181 corresponds to the mass of phosphothreonine (m/z 469.2–288.2). The fragments caused by the loss of phosphoric acid by β-elimination in the gas phase are indicated by horizontal arrows. The m/z 371.2 fragment ion originates from the β-elimination in the gas phase of the phosphopeptide pTIR generating 2-amino-dehydrobutyric acid bound to IR, the latter characterized by the m/z 83 mass difference (371.2–288.2) [18] further confirming the presence of the phosphate group on the threonine residue.

3.4. Chk1-mediated CK2β phosphorylation at Thr213 does not affect Chk1–CK2β complex formation but it does a Chk1 kinase-dead mutant

The identification of Thr213 as CK2β residue phosphorylated by Chk1 kinase was verified by site directed mutagenesis. A recombinant CK2β mutant was created where Thr213 was exchanged for Ala (CK2β T231A). The mutated protein was expressed and purified to homogeneity for in vitro phosphorylation assays in the presence of recombinant Chk1 kinase. As indicated in Fig. 5A, only CK2β wild-type could be extensively phosphorylated by Chk1 (Fig. 5A, lane 4) but not CK2β T213A (Fig. 5A, lane 5). Next, we verified the result described above using native Chk1 kinase as a kinase source. Lysates from transfected cells with HA-Chk1 plasmid were subjected to immunoprecipitation with a polyclonal anti-HA antibody. The immunoprecipitated Chk1 was then utilized as a kinase source in an in vitro kinase assay. As shown in Fig. 5B, only CK2β wild-type could be efficiently phosphorylated by native Chk1 (Fig. 5B, lane 2), while no significant phosphorylation was observed with CK2β T231A mutant (Fig. 5B, lane 3). The fact that CK2β is efficiently phosphorylated by Chk1 kinase led us to verify whether the phosphorylation status of CK2β played a role in the association between the two proteins. Cos-1 cells were co-transfected with HA-Chk1 and CK2β-MycHis or with HA-Chk1 and CK2β T213A-MycHis. Co-immunoprecipitation experiments revealed that the phosphorylation of CK2β at Thr213 is not necessary to allow the complex formation (results not shown). Interestingly, immunoprecipitation experiments performed with a polyclonal anti-CK2β antibody on lysates of Cos-1 cells co-transfected with either

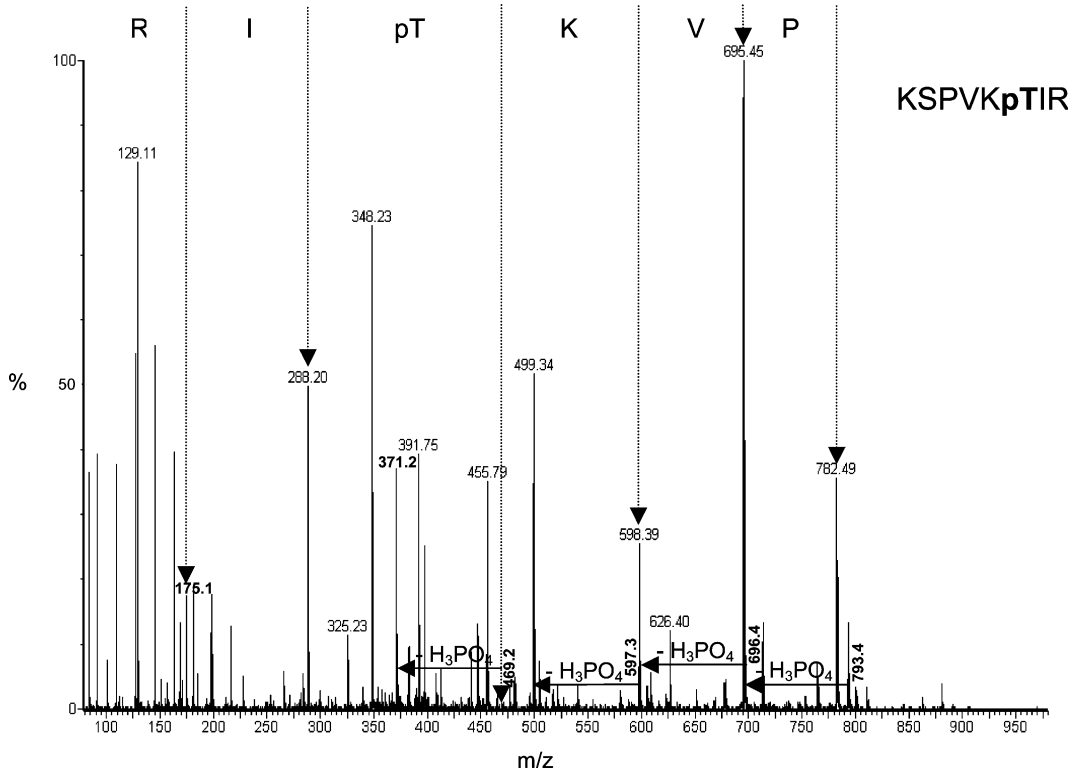

Fig. 4. The MS/MS spectrum of the double charged m/z 504.77 precursor ion corresponding to the singly phosphorylated peptide 208-KSPVKTIR-215. The punctuated vertical arrows indicate the singly charged fragment ions and the amino acids between the punctuated vertical arrows verify the sequence of the peptide. The horizontal arrows indicate the loss of H_3PO_4 (−98 Da).

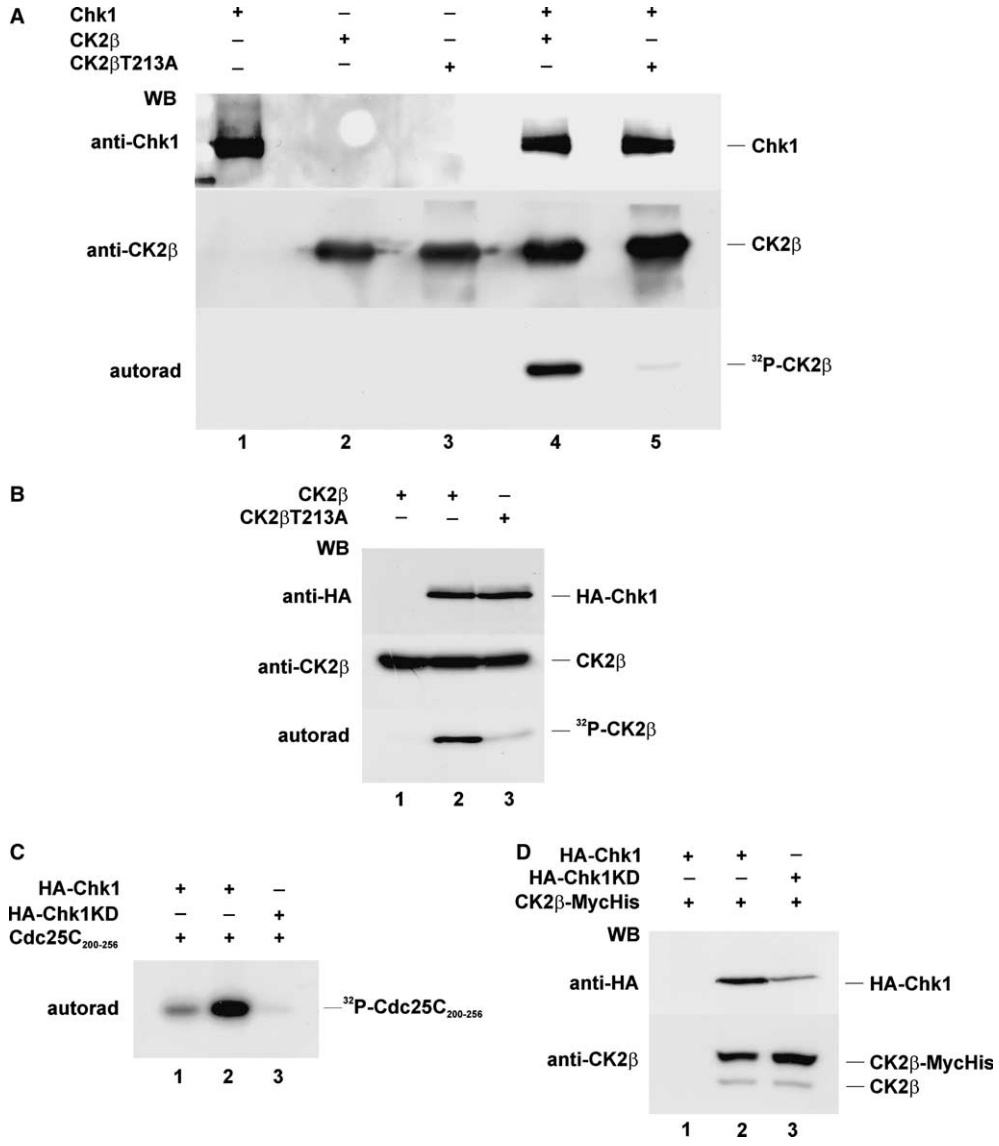

Fig. 5. Phosphorylation of CK2β at Thr213 is not required for the formation of a stable complex with Chk1. (A) In vitro kinase assay of CK2β (lanes 2 and 4) and CK2β T213A (lanes 3 and 5) in the presence of recombinant GST-Chk1 kinase. (B) Cos-1 cells were transiently transfected with HA-Chk1 plasmid. Lysates were subjected to immunoprecipitation with polyclonal anti-HA antibody (lanes 2 and 3) or a control serum (lane 1). The immunoprecipitated Chk1 was used as a kinase source for an assay in the presence of recombinant CK2β wild-type (lanes 1 and 2) or CK2β T231A (lane 3) proteins. (C) Cos-1 cells were transfected with HA-Chk1 (lanes 1 and 2) or an inactive HA-Chk1 mutant (HA-Chk1KD, lane 3). Cell lysates were subjected to immunoprecipitation with polyclonal anti-HA antibody (lanes 2 and 3) or a control serum (lane 1). The precipitated Chk1 kinase was tested in an in vitro kinase assay using as a substrate GST-Cdc25C$_{200–256}$. (D) Protein extracts from cells transfected with HA-Chk1 and CK2β-MycHis or HA-Chk1KD and CK2β-MycHis plasmids were subjected to immunoprecipitation with rabbit polyclonal anti-CK2β antibody (lanes 2 and 3) or a control serum (lane 1). In all the experiments, proteins separated by SDS–PAGE and subsequently transferred to PVDF membrane were exposed to film and detected with specific antibodies.

wild-type HA-Chk1 and CK2β-MycHis or with a kinase-inactive mutant of Chk1 (HA-Chk1KD, Fig. 5C, lane 3) and CK2β-MycHis, revealed that the ectopically expressed HA-Chk1KD leads to a less stable interaction with CK2β (Fig. 5D, lanes 2 and 3).

4. Discussion

Protein kinase CK2 is a highly conserved enzyme involved in many metabolic processes although its true intracellular role remains unsolved and controversial. It is an essential kinase in yeast [19] and its dysregulated expression in transgenic mice contributes to lymphocyte transformation [20], supporting the idea that increased CK2 kinase activity correlates with tumor development. Despite the fact that in vivo and in vitro CK2 exists mainly as a holoenzyme, increasing evidences suggest that the subunits exist in vivo as isolated entities exerting specific functions in complex with other cellular partners. Undoubtedly, the regulatory β-subunit of CK2 plays a role in many cellular events as it has been demonstrated by genetic studies in *S. cerevisiae* and *S. pombe* (reviewed by [2]), in Xeroderma pigmentosum cell line [21] and in transgenic mice where, in contrast to what occurs in yeast, CK2β is essential

for cell viability in early embryonic development [22]. Recently, we were able to show that CK2β interacts with the human cell cycle checkpoint kinase Chk1 [7] enhancing its kinase activity. In this study, we have shown that Chk1 specifically phosphorylates CK2β, as isolated subunit, at Thr213 although at present we cannot exclude the presence of other minor site(s). The fact that CK2 holoenzyme does not constitute a substrate target for Chk1 demonstrates that the CK2β phosphorylation site at Thr213 is located in a structurally inaccessible domain. Indeed, a careful analysis of CK2 tertiary structure reveals that the CK2α/β contacts in the rhCK2 complex occur twice: through the so-called CK2β body domain of one rhCK2β molecule and the so-called CK2β tail (C-domain) of the other rhCK2β subunit [5]. It is worth to mention that the same CK2β C-terminal domain, where Thr213 resides, is also the one involved in the complex formation with Chk1 and that different domains of CK2β have been shown to be involved in protein-protein interactions. It remains to be determined whether, in the sequence of CK2β, these binding regions interact with different proteins having a regulatory significance.

As shown in Section 3, the complex between CK2β and Chk1 is not affected by the modification of CK2β at Thr213. On the other hand, our results clearly show that a stable complex formation requires the integrity of Chk1 kinase and that the stability of the complex in intact cells may be under the control of a conformationally active Chk1. Based on the present data, a dual role of CK2β could be suggested: as a substrate target of Chk1 which accounts for the competition with other Chk1 substrates (i.e., Cdc25C) and as an activator of Chk1 kinase presumably when in complex with the kinase. The fact that under checkpoint activation there is no change in Chk1–CK2β complex formation and that the CK2β-associated Chk1 has considerable higher kinase activity [7], suggests that the basal activity of Chk1 might be dependent on its interaction with CK2β. One could speculate that defective Chk1 proteins, where Chk1–CK2β complex formation is attenuated or impaired, might contribute to enhance genetic instability in some tumors where cancer cells are less prone to activate checkpoint signaling and to trigger apoptosis. At present, we cannot exclude that Chk1-catalyzed CK2β phosphorylation might affect the interaction with other cellular proteins. A careful examination of this complex during different phases of the cell cycle and the search for additional interacting partners will help to better define the biological significance of Chk1–CK2β complex in further studies.

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